Altered connectivity between cerebellum, visual, and sensory-motor networks in Autism Spectrum Disorder

Altered Connectivity Between Cerebellum, Visual, and Sensory-Motor Networks in Autism Spectrum Disorder: Results from the EU-AIMS Longitudinal European Autism Project

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Abstract

Background: Resting-state fMRI-based studies on functional connectivity in autism spectrum disorder (ASD) have generated inconsistent results. Interpretation of findings is further hampered by small samples and a focus on a limited number of networks, with networks underlying sensory processing largely under-examined. We aimed to comprehensively characterize ASD-related alterations *within* and *between* 20 well-characterized resting-state networks using baseline-data from the EU-AIMS Longitudinal European Autism Project.

Methods: Resting-state fMRI data was available for 265 individuals with ASD (7.5-30.3 years; 73.2% male) and 218 typically developing (TD) individuals (6.9-29.8 years; 64.2% male), all with IQ>70. We compared functional connectivity within 20 networks –obtained using independent component analysis– between the ASD and TD group, and related functional connectivity within these networks to continuous (overall) autism trait severity scores derived from the Social Responsiveness Scale-2 across all participants. Furthermore, we investigated case-control differences and autism trait-related alterations in between-network connectivity.

Results: Higher autism traits were associated with increased connectivity *within* salience, medial motor, and orbitofrontal networks. However, we did not replicate previously reported case-control differences within these networks. The *between*-network analysis did reveal case-control differences showing on average 1) decreased connectivity of the visual association network with somatosensory, medial and lateral motor networks, and 2) increased connectivity of the cerebellum with these sensory and motor networks in ASD compared to TD.

Conclusions: We demonstrate ASD-related alterations in within and between-network connectivity. The between-network alterations broadly affect connectivity between cerebellum, visual, and sensory-motor networks, potentially underlying impairments in multisensory and visual-motor integration frequently observed in ASD.

Introduction

Autism Spectrum Disorder (ASD) is an early-onset neurodevelopmental condition affecting 1 to 2% of people worldwide (1). Core behavioral symptoms are impairments in social interaction and communication, the presence of repetitive and restrictive stereotypic behaviors and interests, and atypical sensory processing (2). Yet, symptom presentation and severity vary widely among diagnosed individuals. One key hypothesis is that the diverse symptoms observed in ASD are associated with atypical interactions across distributed brain networks rather than alterations in isolated brain regions (3). This hypothesis is supported by initial task-based functional magnetic resonance imaging (fMRI) studies demonstrating reduced functional connectivity in ASD, suggesting global or long-range hypo-connectivity in ASD (4, 5).

However, more recent studies using resting-state fMRI (R-fMRI) to investigate group differences in functional connectivity yielded more heterogeneous findings (for reviews, see 6, 7, 8). While several case-control studies reported reduced connectivity in ASD, for example between insula and amygdala (9) or within the default mode network (DMN; 10, 11), others demonstrated increased subcortical-cortical connectivity (12, 13) or increased connectivity within DMN, salience, motor, and visual networks in ASD (14). Given that increased connectivity was more frequently reported in childhood ASD and decreased connectivity more frequently in adulthood ASD, these findings were initially ascribed to developmental effects around puberty (15). Yet, this hypothesis does not accommodate more recent studies reporting functional connectivity increases in certain brain regions, but decreases in other areas in both children with ASD (16, 17) and adults with ASD (18). These studies indicate that hypotheses of a global increase or decrease in connectivity are likely overly simplistic and that functional connectivity changes in ASD might be network-dependent. However, most R-fMRI studies have focused on a limited number of networks, investigating for example only the DMN (19, 20) or salience network (21). While contributing to the knowledge of connectivity alterations in ASD, this narrow focus makes it difficult to determine

whether observed ASD-related alterations are indeed specific to the networks investigated or reflect a more global change in connectivity. In addition, other methodological differences between studies, such as the use of ICA-based vs seed-based approaches and the applied motion correction strategy, might also have contributed to the heterogeneity in findings (6).

More importantly, only few studies have examined connectivity *between* different networks in ASD (13, 22). Between-network connectivity reflects the integration of information between different networks, which is vital for many functions including perception, learning, and performing complex cognitive functions, such as social interaction and communication (23, 24). The investigation of between-network connectivity might thus reveal important insights into the functional architecture underlying ASD. Indeed, it was recently demonstrated that connectivity between sensory networks and a subcortical and cerebellar network was increased in ASD compared to controls (13). While networks underlying sensory processing are relatively under-examined in ASD –likely because atypical sensory processing was only recently added to the *DSM-5* diagnostic criteria (2)– these findings highlight the potential significance of between-network connectivity alterations in ASD.

In the present study, we investigated functional connectivity alterations in ASD across the entire brain in 478 participants of the EU-AIMS Longitudinal European Autism Project (LEAP; 25). Using this large, multicenter dataset including individuals with ASD and typically developing (TD) controls across a wide age range (6.9-30.3 years), we aimed to provide a comprehensive, data-driven characterization of ASD-related functional connectivity alterations both *within* and *between* 20 different resting-state networks (RSNs). RSNs were obtained using independent component analysis (ICA; 26) and covered the whole brain, including the sensory networks. Finally, to better capture the phenotypic heterogeneity among ASD and TD participants, we not only compared functional connectivity between the categorically-defined ASD and TD group, but also conducted dimensional analyses relating functional connectivity to a continuous measure of autism trait severity across all participants.

Methods & Materials

Participants

Participants were part of EU-AIMS LEAP, a large multi-center European initiative aimed at the identification of biomarkers in ASD (25). The study comprises 437 individuals with ASD and 300 TD individuals, both males and females, aged between 6 and 30 years. Participants underwent comprehensive clinical, cognitive, and MRI assessment at one of the following five centers: Institute of Psychiatry, Psychology and Neuroscience, King's College London, United Kingdom; Autism Research Centre, University of Cambridge, United Kingdom; Radboud University Nijmegen Medical Centre, the Netherlands; University Medical Centre Utrecht, the Netherlands; Central Institute of Mental Health, Mannheim, Germany. The study was approved by the local ethical committees of participating centers and written informed consent was obtained from all participants or their legal guardians (for participants <18 years). For further details about the study design we refer to Loth et al. (25), and for a comprehensive clinical characterization of the LEAP cohort we refer to Charman et al. (25). In the present study, we selected all participants with an IQ>70 for whom a structural and R-fMRI scan were available (N=553). Participants with a brain abnormality (N=13; mostly not clinically relevant), an incomplete R-fMRI scan (N=5; <75% completed), excessive head motion during the R-fMRI scan (N=43; mean root mean squared of the frame wise displacement (meanFD>0.5; 27)), and insufficient brain coverage (N=14) were excluded. This resulted in the inclusion of 265 individuals with ASD and 213 TD individuals in our analyses. The clinical and demographic characteristics of these participants are given in Table 1.

< Insert Table 1>

Clinical measures

Participants in the ASD group had an existing clinical diagnosis of ASD according to the DSM-IV/ICD-10 or DSM-5 criteria. The diagnosis of ASD participants was confirmed using combined information of the Autism Diagnostic Interview-Revised (ADI-R; 29) and Autism Diagnostic Observation Schedule 2 (ADOS-2; 30). We used the total raw score on the Social Responsiveness Scale Second Edition (SRS-2; 31) as a continuous measure for autism traits across all participants since this measure was available for both ASD and TD individuals. The SRS-2 allows assessment of autism traits across clinical and non-clinical samples and includes 65 questions about autistic behaviors, generating scores ranging from 0 to 195, with higher scores indicating more severe impairments. For TD adults we employed the SRS-2 self-report version (as only the self-report was administered to this group), for all other participants the parent-report was available. (In the supplement we show that our findings are not dependent on SRS-2 informant).

Derivation of 20 resting-state networks

Scans were obtained using 3T MRI scanners at the five different sites. Acquisition parameters of the multi-echo R-fMRI scan and structural scan as well as the preprocessing procedure are detailed in the Supplemental material. All analyses described below were conducted in MNI152 standard space.

To investigate functional connectivity alterations, we first extracted 20 spatially independent components by applying ICA (with dimensionality 20) as implemented in FSL melodic (26) to R-fMRI data of 75 TD participants. This TD sample included participants from each site and consisted of 25 children (6.9-11 years), 25 adolescents (12-17 years), and 25 adults (18-30 years) to obtain resting-state components representative for all sites and the full age-range of our sample. The dimensionality was set to 20 to enforce a split of the sensory and motor systems into their primary and secondary components (as shown before by (33)), which enables a more detailed investigation of these systems. Visual

inspection showed that the obtained components did not contain components representing noise, but instead all represented well-known and reproducible resting-state networks (RSNs). This is likely related to the application of careful ICA-based correction for head motion effects (using ICA-AROMA; 34) in our preprocessing pipeline. Accordingly, all 20 components were selected for further analyses. Figure 1 shows the spatial configuration of all RSNs, including sensory, motor, DMN, and task-related networks. The TD subjects used for the derivation of these RSNs were excluded from further analyses. For each of the remaining 138 TD and 265 ASD participants, we applied dual regression as implemented in FSL (35, 36) to obtain the subject-specific spatial maps and mean timeseries (across all voxels in the spatial map) corresponding to the 20 RSNs.

Investigation of within-network connectivity

To examine alterations in within-network connectivity in ASD, we compared the spatial maps of the 20 RSNs between the TD group (N=138) and ASD group (N=265) using a categorical analysis. We also investigated how within-network connectivity changed as a function of autism traits, by examining the relationship between the spatial maps of the 20 RSNs and SRS-2 scores across all ASD and TD participants with SRS-2 scores available (N=358) in a continuous analysis. We conducted these analyses within the general linear modeling framework where we included, next to diagnostic group (categorical analysis) or the SRS-2 score (continuous analysis), nuisance variables for scan site, sex and age. In both analyses we applied permutation testing (with N=10000 permutations) as implemented in FSL randomise (37) to assess statistical significance. We further applied threshold-free cluster enhancement and family-wise error correction. We corrected for testing multiple RSNs using a Bonferroni-corrected *p*-value of $p<0.0025$ (i.e., 0.05/20 RSNs). For the statistical sensitivity of these analyses we refer to the Supplemental material. Please note that we test influences of diagnosis and autism trait scores in separate statistical models given that autism traits are an essential part of the diagnostics of ASD, i.e.,

those participants with very high autism trait scores by definition get an ASD diagnosis. Including them in one model would remove all variance shared between an ASD diagnosis and autism traits, and thus remove a large part of the variance associated with ASD.

< Insert Figure 1 >

Investigation of between-network connectivity

To investigate between-network connectivity, we computed Pearson and partial correlations between mean timeseries of the 20 RSNs obtained for every participant. For both correlation types, this resulted in 190 functional connections (i.e., between-network correlations). As opposed to Pearson correlations, partial correlations represent the association between two networks after accounting for the variance they share with all other networks in the analysis and can thus be interpreted as a measure of direct connectivity between networks. All of the following steps were conducted for both Pearson and partial correlations. The obtained correlations were transformed into normally distributed values using Fisher's *r*-to-*z* transformation for every participant. We then applied an ordinary least squares regression for each correlation to correct for potential confounding effects of scan site, sex, and age. Next, we conducted a categorical analysis comparing between-network connectivity between the ASD and TD group. More specifically, we tested group differences in the residual correlation strength for significance by means of permutation testing (with N=10000 permutations) for every network-pair. *P*-values were obtained by calculating the fraction of permutated samples that yielded a group-difference larger than the observed difference. In addition, we conducted a continuous analysis in which we investigated the relationship of between-network connectivity with SRS-2 scores across all participants. *P*-values were obtained by calculating the fraction of permutated samples that yielded a correlation of SRS-2 scores with between-network connectivity higher than the observed correlation. In both the categorical and

continuous analyses we corrected for multiple comparisons by applying a False Discovery Rate (FDR) correction (q<0.05).

Post-hoc analyses

First, we conducted the continuous SRS-2 analyses in the ASD and TD group separately to ensure that observed associations across all participants did not simply reflect a mean group difference in connectivity. In addition, we repeated all our analyses in children, adolescents and adults separately to investigate whether additional connectivity alterations were revealed by investigating each age-group independently, in light of potential developmental effects. We also checked whether the significant connectivity alterations identified in our analyses were specific to a particular ASD symptom domain. To this end, we examined post-hoc correlations with the Short Sensory Profile (SSP; 38), and with the Social Communication and Interaction (SCI) and Restrictive interests and Repetitive Behavior (RRB) subscales of the SRS-2. Finally, we conducted sensitivity analyses to rule out that significant connectivity alterations were accounted for by head motion, informant (parent or self-report SRS-2 score for the continuous analyses), sex, scan site, IQ, medication use, or comorbidity with attention-deficit/hyperactivity disorder (ADHD). All post-hoc analyses are detailed in the Supplemental material.

Results

Autism trait-related alterations in within-network connectivity

Comparing the spatial maps of the 20 RSNs between the ASD and TD group did not reveal a main effect of diagnosis on functional connectivity within any of the 20 RSNs. However, the analysis in which we investigated continuous effects of autism traits by relating functional connectivity within the 20 RSNs to SRS-2 scores across all participants revealed significant associations for three networks (Figure 2). More specifically, we observed that functional connectivity increased with higher SRS-2 scores (i.e., more severe autism traits) within the anterior salience network (cluster in superior frontal gyrus (SFG)), medial motor network (large cluster extending to SFG), and orbitofrontal cortex (OFC) network. These autism trait-related connectivity alterations were also significant when only investigating the ASD group – ensuring that these are not artificial correlations induced by a general difference in the mean of the ASD and TD group (Table S1, Figure S1)– and correlated with atypical sensory processing, repetitive behaviors and social impairments (Table S2). Post-hoc analyses further showed these connectivity alterations were not related to head motion, IQ, sex, scan site, age, medication use, comorbidities or SRS informant.

Case-control and autism trait-related alterations in between-network connectivity

Next, we investigated ASD-related alterations in functional connectivity between the 20 RSNs. As shown in Table 2 and Figure 3, the categorical analysis revealed that Pearson correlations of 16 edges (i.e., functional connections between networks) differed significantly between the ASD and TD group. Notably, 10 of the 16 significant edges included the visual association network or cerebellar network. Compared to the TD group, functional connectivity was decreased in the ASD group between visual association, somatosensory, medial motor and lateral motor networks. At the same time, functional connectivity of the cerebellum with all these sensory and motor networks was increased in ASD.

Furthermore, post-hoc analyses showed that connectivity for several of these edges was associated with atypical sensory processing, repetitive behaviors and/or social impairments (Table S3). We observed no significant group differences in the partial correlation analyses.

In the continuous SRS-2 analysis, we observed significant associations for four network pairs: at higher SRS scores, connectivity (i.e., Pearson correlations) of the cerebellum with the somatosensory and medial motor network increased, whereas connectivity of the OFC with the lateral motor network and posterior DMN decreased (Table 2, Figure 3). Marginally significant correlations with the SRS (r>±0.15, p<0.05; see Figure 3) were present for the visual association edges implicated in the categorical analysis, however these did not survive FDR-correction. Also, the partial correlation analyses did not show significant between-network alterations.

Post-hoc analyses confirmed that none of the significant categorical or continuous ASD-related alterations in between-network connectivity were accounted for by head motion, IQ, sex, scan site, medication use, or comorbidities; although some of the between-network connectivity differences were smaller or not yet present in childhood, warranting further investigation into the development of between-network connectivity in ASD (see Supplemental pages 10-11).

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Discussion

We conducted a comprehensive investigation of ASD-related differences in within- and between-network functional connectivity in the large and clinically well-characterized EU-AIMS LEAP cohort. The key findings of our study are the differences observed in between-network connectivity: while connectivity between visual association, somatosensory, and motor networks was decreased, connectivity of the cerebellum with all these sensory and motor networks was increased in the ASD compared to TD group. Furthermore, we observed that at higher autism trait severity connectivity increased within the anterior salience, medial motor, and OFC networks. However, we did not replicate previously reported case-control differences in within-network connectivity.

Impaired multisensory and visual-motor integration in ASD

We propose that the decreased functional connectivity between visual association, somatosensory and motor networks underpins the abnormalities in multisensory and visual-motor integration observed in individuals with ASD. Over the last decade, a growing literature has reported atypical sensory processing in ASD, such as hypo- or hyper-reactivity to sensory stimuli, enhanced sensory discrimination, and impaired multisensory integration (39, 40). Moreover, the *DSM-5* now includes atypical sensory processing as a diagnostic criterion (2), acknowledging the significance of these alterations in ASD. Similarly, evidence is emerging for impaired visual-motor integration in ASD (41, 42). For example, children with ASD favor proprioceptive over visual feedback when learning novel movements (43), have difficulty incorporating visual input into movement planning (41) and show decreased performance on visual-motor coordination tasks (44). Our findings nicely concord with degree centrality-based (a graph theory metric indicating the connectedness of voxels) analyses of the EU-AIMS LEAP cohort, showing reduced connectedness of sensory and motor areas in the brain (Holiga et al., in revision). We also replicate a previous R-fMRI report showing decreased connectivity between the visual association and

lateral motor network in ASD (45). The multitude of functional visual-motor and visual-sensory connections affected in the ASD group as well as their association with not one specific, but multiple ASD symptom domains observed in our analysis, suggests that impaired visual-motor and multisensory integration –while relatively under-examined– could play a very central role in ASD. Indeed, visual-motor and multisensory integration are crucial for developing imitation skills, and important for learning motor, communication and social skills (46, 47). Impairments in these skills comprise the core symptoms of ASD. Accordingly, and in accordance with Nebel et al. (45, 48), we hypothesize that our findings reflect impaired visual-motor and multisensory integration and may represent fundamental abnormalities underpinning various symptoms in ASD.

The increased connectivity of the cerebellum with seven cortical networks in the ASD group can be interpreted within the larger framework of structural and functional cerebellar alterations that frequently have been reported in ASD (for reviews, see 49, 50). It is striking that most of the networks with which the cerebellum exhibited increased connectivity in ASD are again the networks underlying sensory and motor processing. While initially considered a motor region, various studies have now established an important role for the cerebellum in multi-modal integration (51-53), suggesting that also hyperconnectivity of cerebellum with sensory and motor networks might be associated with impaired multisensory and sensory-motor integration. Our findings are consistent with work from Cerliani et al. (13) showing increased functional connectivity between the crus region of cerebellum and a network including dorsal motor and somatosensory cortices in ASD (though we did not replicate the increased cortico-striatal/thalamic connectivity observed in their study) and with the increased cerebro-cerebellar connectivity reported by Khan et al. (54). Our findings are also in accordance with previously reported abnormalities in structural connectivity of cerebellar outputs (55). The increased functional connectivity of cerebellum observed in our study might relate to the frequently reported reduction in GABAergic Purkinje cells in ASD (56, 57). These cerebellar neurons send inhibitory projections to the deep

cerebellar nuclei, the output nuclei of the cerebellum. Loss of these neurons is thought to lead to disinhibition of the deep cerebellar nuclei (13, 58), which could explain the observed shift from negative to positive (i.e., increased) cerebro-cerebellar connectivity in the ASD group. While both decreased connectivity of the visual association network and increased connectivity of the cerebellum with sensory and motor networks strongly point to impaired multisensory and visual-motor integration in ASD, further research is necessary to determine how exactly these findings can be integrated.

Autism trait-related increases in within-network connectivity

In our within-network analysis we observed that at higher autism traits functional connectivity increased within the anterior salience, medial motor, and OFC networks –across the sample as a whole and within the ASD group separately– and correlated with atypical sensory processing, repetitive behaviors and social impairments. These networks correspond with networks implicated in previous case-control studies in ASD. For example increased connectivity within the salience network has been reported by R-fMRI studies before in ASD (14, 59). The salience network is thought to be involved in selecting which of many internal and external stimuli one should pay attention to (60, 61) and alterations in this network have been associated with hypersensitivity in ASD (21). Increased connectivity within the medial motor network has also been reported in ASD (14, 62) and has been related to impairments in motor function in ASD (63). We further observed increased connectivity within the OFC network. Aberrant structure and function of the OFC has been observed in ASD before and has been related to social impairments in ASD (64-66). In contrast to previous reports (19, 20), we did not observe altered connectivity in the DMN in this analysis, yet the between network-analysis revealed increased connectivity between two subnetworks of the DMN (the posterior DMN and PCC network). It is further apparent that all symptom-related alterations observed in our analysis are increases in functional connectivity whereas previous studies have also reported decreased within-network connectivity in ASD. In light of potential

developmental effects, we checked for the influence of age on our findings. Post-hoc analyses however showed that all ASD trait-related increases in connectivity were present across children, adolescents and adults (Table S4).

Although we demonstrated that connectivity within multiple networks was increased at higher SRS-2 scores in the continuous analyses (which allows for larger individual variation), it is noteworthy that we did not replicate previously observed case-control differences in within-network connectivity (e.g., 9, 11-14). A factor that might have contributed to the absence of significant categorical differences in within-network connectivity in our study is the heterogeneity in our large sample. The LEAP study specifically aimed to include a broad sample of individuals with ASD to provide a valid representation of the general (i.e., real-word) ASD population: participants across the entire autism spectrum were selected independent of sex and within a large age range. This approach might have concealed case-control differences in within-network connectivity detected in previous studies, which were mostly conducted in smaller and/or matched samples that were potentially more homogeneous. However, our sample likely reflects the actual heterogeneity present in ASD, while findings from previous studies using smaller, more homogeneous samples might not always generalize to the entire ASD population.

That being said, our significant findings should also be interpreted in the context of the large heterogeneity in ASD. As can be observed in Figure 2, inter-subject variability is high and not all subjects with high ASD severity scores display high connectivity within the respective networks. Similarly, despite the significant case-control differences in between-network connectivity, effect sizes were small to medium (Table 2) and boxplots of these effects show substantial overlap between groups (Figure S3). This indicates that while –on average– connectivity for these functional connections is altered in the ASD group, these alterations are not present in all individuals with ASD. This heterogeneity in ASD is often overlooked by the R-fMRI literature. Future work of the EU-AIMS LEAP consortium will focus on defining

ASD subtypes based on the underlying connectivity profile (67) and normative modeling approaches (68), which will be key into further unraveling the potentially heterogeneous neurobiological mechanisms underlying ASD.

Finally, post-hoc analyses did not reveal other significant ASD-related connectivity alterations when conducting our analyses in children, adolescents and adults separately, in addition to the alterations that were already observed in our main analyses. However, some of the between-network (but not within-network) connectivity alterations in our main analyses, were not present or of smaller magnitude in children compared to adolescents and adults. This implies potential effects of development affecting between-network connectivity. With the follow-up assessment of LEAP cohort nearly completed, future work will include a longitudinal analysis to assess the precise effects of development on functional connectivity in ASD.

A limitation of our study is that while our findings strongly implicate impaired multisensory and visual-motor integration in ASD, the LEAP cognitive task-battery did not include assessments of these domains, so future work will be necessary to confirm the direct link between aberrant between-network connectivity and impaired visual-motor and multisensory integration in ASD. Other limitations are that the continuous SRS-2 analysis was based on self-report scores for adult TD individuals compared to parent-report scores for all other participants, and that the ASD and TD group significantly differed in the proportion of males and females, head motion and IQ. However, sensitivity analyses revealed no influence of these factors on our findings.

Conclusion

We demonstrate widespread alterations in functional connectivity between visual, cerebellum and sensory-motor networks in ASD compared to controls, implicating a key role for impaired multisensory and visual-motor integration in ASD.

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Figure legends

Figure 1. The 20 resting-state networks (RSNs) obtained using independent component analysis (ICA). All RSNs were used for further analyses. *Z*-stat maps are thresholded at *Z*>3.1 and shown in radiological convention.

Figure 2. Significant autism trait-related alterations in within-network connectivity. Increased Social Responsiveness Scale (SRS-2) scores were associated with increased functional connectivity within three resting-state networks (RSNs). Left panels depict the continuous relationships between SRS-2 scores and connectivity strength within each of the significant clusters. Connectivity strength represents the mean Z-stat value within the significant cluster for every subject. These Z-stat values were derived from the subject-specific spatial maps generated by the dual regression approach (i.e., the GLM parameter estimate statistical image normalised by the residual within-subject noise) corresponding to the ICA-template networks. Data points from TD individuals are indicated in blue and data points from ASD individuals in green. Right panels show the significant clusters (red-yellow) overlaid on the respective RSNs (green; radiological convention). OFC=orbitofrontal cortex.

Figure 3. Between-network connectivity matrices. The top two matrices represent the group-average between-network connectivity matrices of the TD and ASD group. The bottom left matrix represents the difference in between-network connectivity between the ASD and TD group, the bottom right matrix shows the correlation of Social Responsiveness Scale Second Edition (SRS-2) scores with between-network connectivity (i.e., the correlation of SRS-2 scores with Pearson or partial correlations between the timeseries of the 20 RSNs). *Z*-transformed Pearson correlations are shown in the upper right triangle, *Z*-transformed partial correlations in the bottom left triangle of each matrix. Asterisks (*) indicate

significant group differences (top and bottom left) or significant correlations with the SRS score (bottom right); FDR corrected, q<0.05. The statistical parameters of these significant ASD-related alterations are listed in Table 2. Network abbreviations: Visual1=primary visual, Visual2=visual association, Occ.Pole=occipital pole, Motor1=medial motor, Motor2=lateral motor, Somatosens= somatosensory, ant.DMN=anterior default mode, post.DMN=posterior default mode, PCC=posterior cingulate cortex, ant.Salience=anterior salience, post.Salience=posterior salience, left FPN=left fronto-parietal, right FPN=right fronto-parietal, Temp.Par=temporo-parietal, OFC=orbitofrontal cortex, DAN=dorsal attention network.

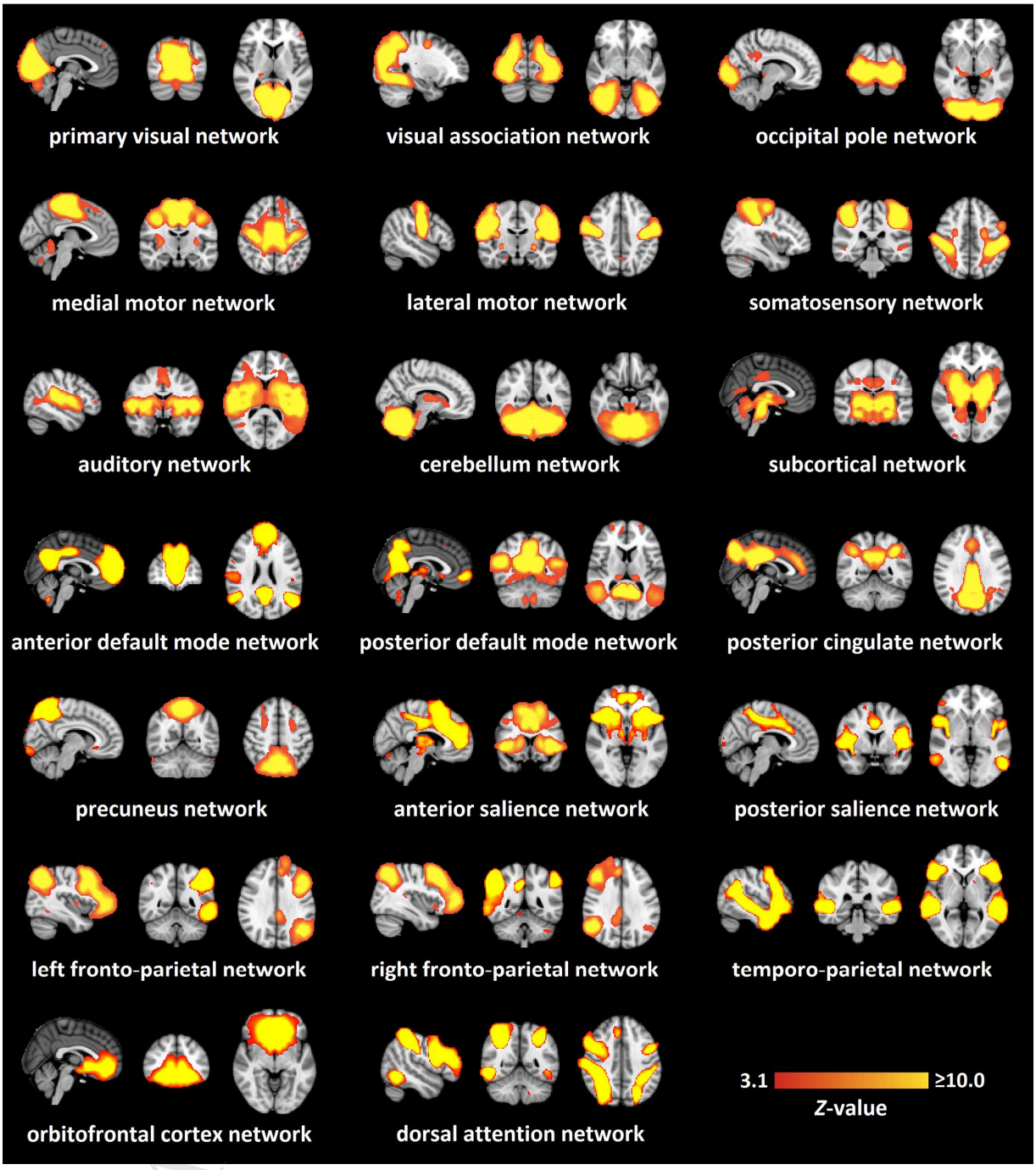
Tables

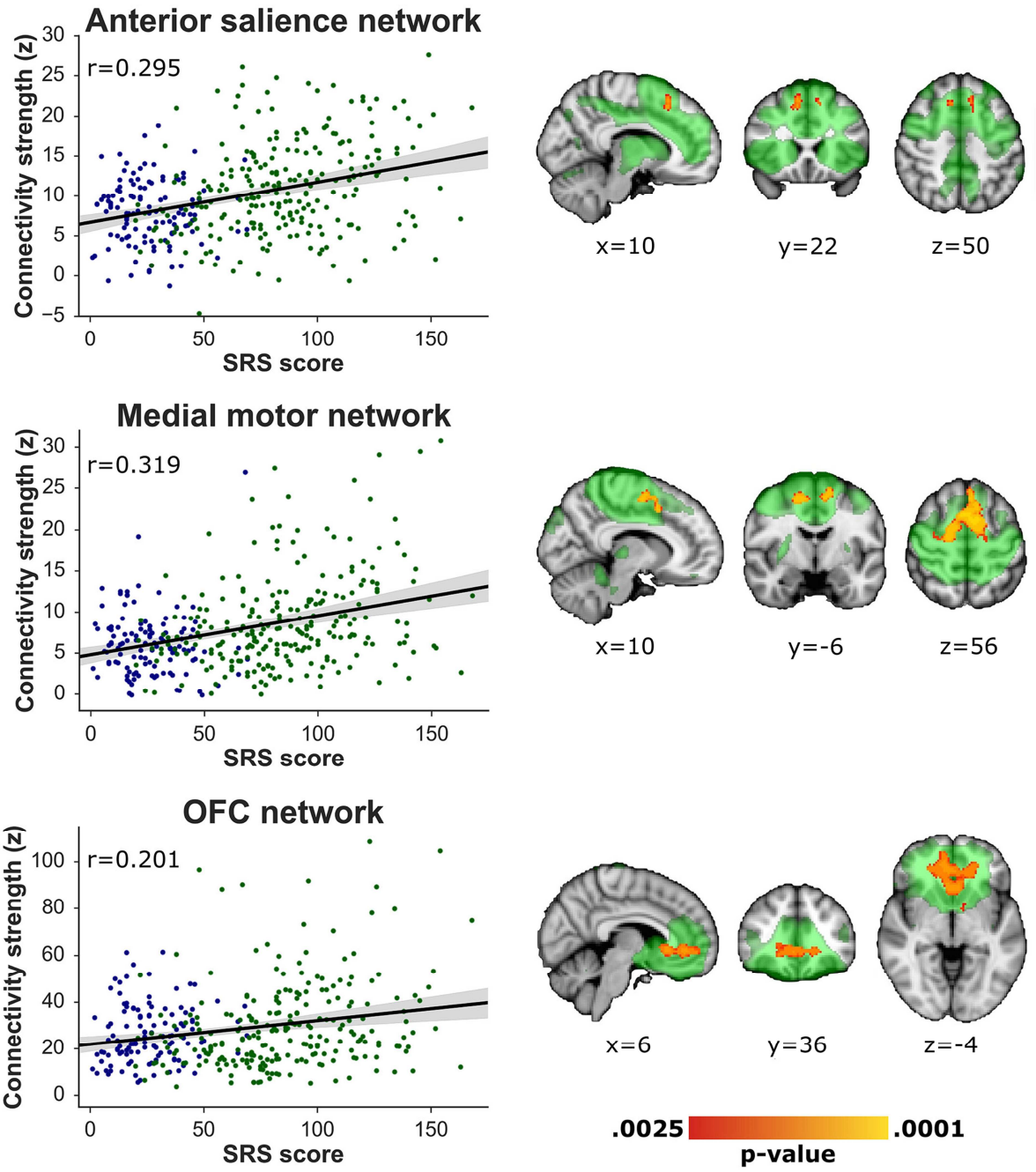
	ASD N=265		TD N=213		Test Statistic	
Demographic (Mean, SD)						
Age, *years*	16.91	*5.43*	17.04	*5.53*	t(476)=-0.272, *NS*	ASD=TD
Full scale IQ	103.67	*16.14*	108.14	*14.20*	t(476)=-3.172, *p*=0.006	ASD<TD
Head motion during R-fMRI scan (meanFD) [a]	0.11	*0.08*	0.084	*0.07*	t(476)=3.024, *p*=0.009	ASD>TD
Demographic (Number, %)						
Sex, *male*	194	*73.21%*	136	*63.85%*	$X^2(1)$=4.531, *p*=0.038	ASD>TD
Handedness, *right-handed* [b]	145	86.83%	187	82.02%	$X^2(2)$=2.273, *NS*	
Current medication use [c]	68	28.75%	3	2.50%		
Clinical (Mean, SD)						
ADI-R [d]						
Social interaction	16.25	*6.79*	N/A			
Communication	13.14	*5.57*	N/A			
RRB	4.22	*2.69*	N/A			
ADOS-2 [e]						
Social Affect	5.78	*2.60*	N/A			
RRB	4.57	*2.64*	N/A			
Total	5.01	*2.74*	N/A			
SRS raw score [f]	86.13	30.99	24.78	14.49	t(423)=24.85**	ASD>TD
SRS T score [f]	69.22	12.22	45.91	5.23	t(423)=24.23**	ASD>TD
ADHD inattentive symptoms [g]	4.04	*3.14*	0.87	*1.70*	t(405)=9.14**	ASD>TD
ADHD hyper/imp symptoms [g]	2.36	*2.66*	0.38	*1.12*	t(405)=11.91**	ASD>TD

Table 1. Participant characteristics. [a] Motion as measured by the root mean squared of the mean frame-wise displacement (meanFD; 27). [b] Handedness was assessed with the short version of the Edinburgh Inventory (28). Handedness information was available for 167 control participants (left: N=17, ambidexter: N=5, right: N=145) and 228 ASD participants (left: N=32, ambidexter: N=9, right: N=187). [c] Number of participants taking medication prescribed for behavioral or neurological problems. Medication data was available for 238 ASD and 119 TD participants. [d] Autism Diagnostic Interview-Revised (ADI-R; 29). Scores were computed for reciprocal interaction (social interaction), communication, and restrictive, repetitive stereotyped behaviors and interests (RRB). ADI-R scores were available for 253 ASD participants. [e] Autism Diagnostic Observation Schedule 2 (ADOS-2; 30). Calibrated severity scores were computed for social affect (SA), restricted and repetitive behaviors (RRB) and the overall total score. ADOS scores were available for a 233 ASD participants. [f] Total raw and total T score (sex+age normalized) on the Social Responsiveness Scale-2 (SRS-2; 31). SRS-2 scores were available for 416 participants. The raw SRS-2 scores were used in our analyses. [g] ADHD symptoms were assessed with the DSM-5 ADHD rating scale, covering inattention and hyperactivity/impulsivity (hyp/imp) symptoms (32; available for 237 ASD and 170 TD participants). *NS*=not significant; ***p*<0.001.

Network 1	Network 2	Mean correlation (z) TD	Mean correlation (z) ASD	Permuted *p*-value	FDR-corrected *p*-value	Effect size *Cohen's D*
Connectivity decrease in ASD group						
Visual association	Somatosensory	3.9502	2.5368	0.0030	0.0475	-0.3116
Visual association	Motor medial	1.7351	0.3029	0.0002	0.0228	-0.3819
Visual association	Motor lateral	2.7085	1.1762	0.0006	0.0228	-0.3801
Motor lateral	Motor medial	7.4314	5.9793	0.0025	0.0432	-0.3215
Motor lateral	Somatosensory	4.5549	4.1193	0.0034	0.0475	-0.3022
Cerebellum	Subcortical	1.7674	0.2160	0.0011	0.0317	-0.3520
OFC	Motor lateral	-0.4978	-1.5683	0.0015	0.0317	-0.3470
Connectivity increase in ASD group						
Cerebellum	Somatosensory	-0.1909	0.9704	0.0006	0.0228	0.3526
Cerebellum	Motor medial	-1.2208	0.0073	0.0014	0.0317	0.3417
Cerebellum	Motor lateral	-0.7624	0.3023	0.0005	0.0228	0.3480
Cerebellum	Visual association	1.0814	1.9481	0.0035	0.0475	0.3127
Cerebellum	Auditory	-0.2932	0.5087	0.0042	0.0499	0.3005
Cerebellum	Temporal-parietal	0.3017	1.1020	0.0042	0.0499	0.2943
Motor medial	Salience anterior	0.2993	1.4783	0.0014	0.0317	0.3256
DMN anterior	Occipital pole	-0.2438	0.8762	0.0005	0.0228	0.3659
DMN posterior	PCC	7.2185	8.2413	0.0024	0.0432	0.3313
		Correlation with SRS:		**Permuted *p*-value**	**FDR-corrected *p*-value**	**Effect size *Cohen's D***
SRS-related connectivity alteration						
OFC	Motor lateral	-0.229		0.0001	0.0190	N/A
OFC	DMN posterior	-0.169		0.0008	0.0342	N/A
Cerebellum	Somatosensory	0.174		0.0003	0.0190	N/A
Cerebellum	Motor medial	0.186		0.0002	0.0190	N/A

Table 2. Summary of statistical parameters of significant categorical and continuous ASD-related alterations in between-network connectivity. Mean correlations of the TD and ASD group represent group-average *Z*-transformed Pearson correlations. Abbreviations: DMN=default mode network, NA=not applicable, OFC=orbitofrontal cortex, PCC=posterior cingulate cortex, SRS=Social Responsiveness Scale-2.


primary visual network
visual association network
occipital pole network
medial motor network
lateral motor network
somatosensory network
auditory network
cerebellum network
subcortical network
anterior default mode network
posterior default mode network
posterior cingulate network
precuneus network
anterior salience network
posterior salience network
left fronto-parietal network
right fronto-parietal network
temporo-parietal network
orbitofrontal cortex network
dorsal attention network
3.1
≥10.0
Z-value


Anterior salience network
r=0.295
Connectivity strength (z)
SRS score
x=10
y=22
z=50
Medial motor network
r=0.319
Connectivity strength (z)
SRS score
x=10
y=-6
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OFC network
r=0.201
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SRS score
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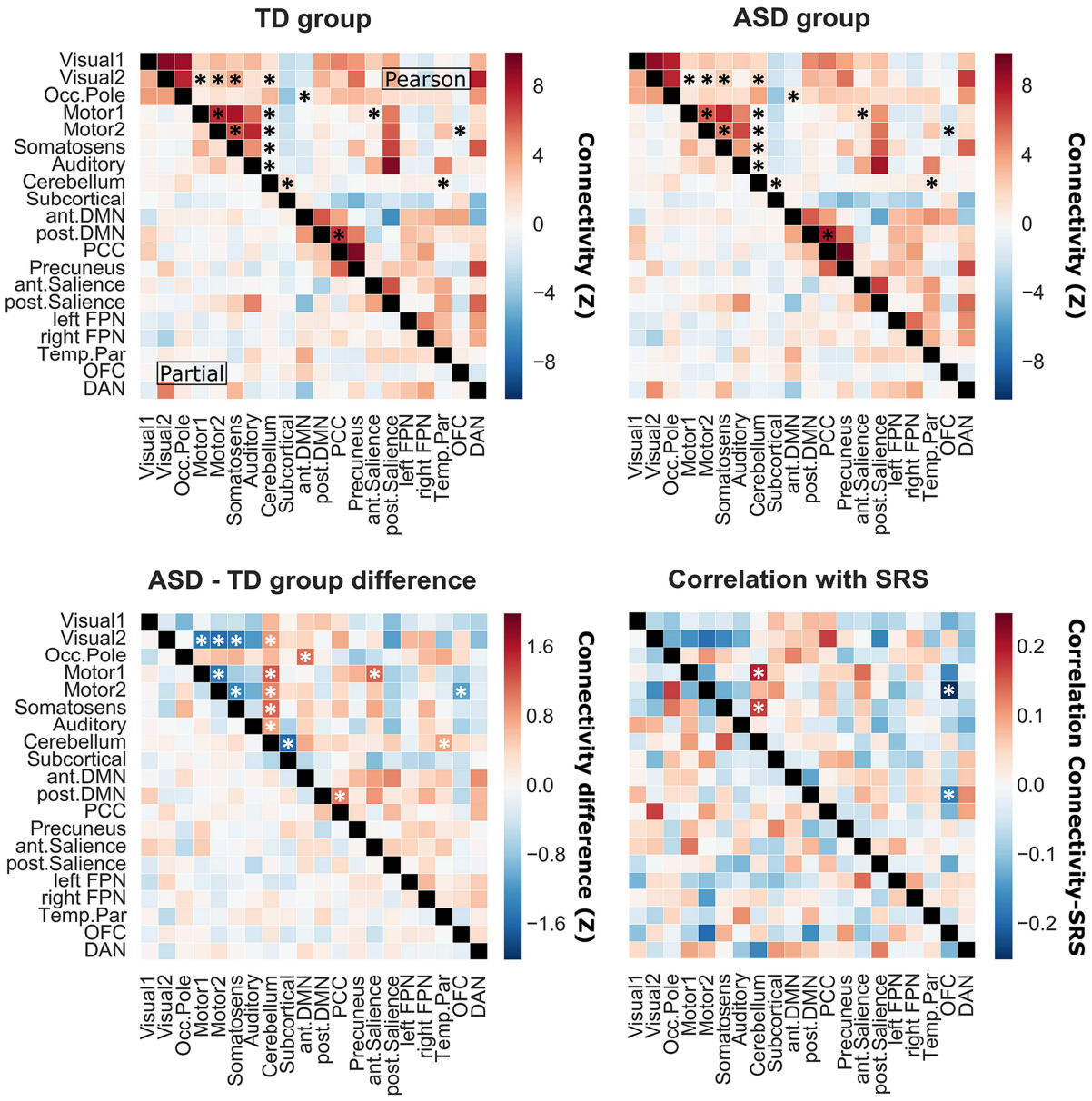
y=36
z=-4
.0025
.0001
p-value


TD group
ASD group
ASD - TD group difference
Correlation with SRS
Pearson
Partial
Visual1
Visual2
Occ.Pole
Motor1
Motor2
Somatosens
Auditory
Cerebellum
Subcortical
ant.DMN
post.DMN
PCC
Precuneus
ant.Salience
post.Salience
left FPN
right FPN
Temp.Par
OFC
DAN
Connectivity (Z)
Connectivity difference (Z)
Correlation Connectivity-SRS

Altered Connectivity Between Cerebellum, Visual, and Sensory-Motor Networks in Autism Spectrum Disorder: Results from the EU-AIMS Longitudinal European Autism Project

Supplemental Information

Content

1. MRI data acquisition and preprocessing

MRI data were acquired on 3T scanners: General Electric MR750 at Institute of Psychiatry, Psychology and Neuroscience, King's College London, United Kingdom (*KCL)*; Siemens Magnetom Skyra at Radboud University Nijmegen Medical Centre, the Netherlands *(RUNMC)*; Siemens Magnetom Verio at Autism Research Centre at the University of Cambridge, United Kingdom *(UCAM)*; Philips 3T Achieva at University Medical Centre Utrecht, the Netherlands *(UMCU)*; and Siemens Magnetom Trio at Central Institute of Mental Health, Mannheim, Germany *(CIMH)*. Procedures were undertaken to optimize the MRI sequences for the best scanner-specific options, and phantoms and travelling heads were employed to assure standardization and quality assurance of the multi-site image-acquisition (1). Structural images were obtained using a 5.5 minute MPRAGE sequence (TR=2300ms, TE=2.93ms, T1=900ms, voxels size=1.1x1.1x1.2mm, flip angle=9°, matrix size=256x256, FOV=270mm, 176slices). An eight-to-ten minute resting-state fMRI (R-fMRI) scan was acquired using a multi-echo planar imaging (ME-EPI) sequence developed by Kundu et al. (2); TR=2300ms, TE~12ms, 31ms, and 48ms (slight variations are present across centers), flip angle=80°, matrix size=64x64, in-plane resolution=3.8mm, FOV=240mm, 33 axial slices, slice thickness/gap=3.8mm/0.4mm, volumes=200 (*UMCU*), 215 (*KCL, CIMH*), or 265 (*RUNMC, UCAM*). Participants were instructed to relax and fixate on a cross presented on the screen for the duration of the R-fMRI scan.

We selected all participants with an IQ>70 for whom a structural and R-fMRI scan were available (N=553). Participants with a brain abnormality (N=13; mostly not clinically relevant), an incomplete R-fMRI scan (N=5; <75% completed), excessive head motion during the R-fMRI scan (N=43; mean root mean squared of the frame wise displacement (meanFD > 0.5)) and insufficient brain coverage (N=14) were excluded. This resulted in the inclusion of 265 individuals with ASD and 213 TD individuals in our analyses. After recombining the three R-fMRI scan echoes using echo-time weighted averaging, the R-fMRI data were preprocessed using a standard preprocessing pipeline that included tools from the FMRIB Software Library (FSL version 5.0.6; http://www.fmrib.ox.ac.uk/fsl). Preprocessing included removal of the first five volumes to allow for signal equilibration, primary head motion correction via realignment to the middle volume (MCFLIRT; 3), grand mean scaling and spatial smoothing with a 6mm FWHM Gaussian kernel. Next, we thoroughly corrected for secondary head-motion related artifacts, by applying ICA-AROMA, a novel ICA-based method, which automatically detects and removes motion-related components from the data (4). ICA-AROMA has been demonstrated to remove head motion-related artifacts with high accuracy while preserving signal of interest (4, 5). Finally, we applied nuisance regression to remove signal from white matter and cerebrospinal fluid, and a high-pass filter (0.01 Hz). The R-fMRI images of each participant were co-registered to the participants' anatomical images via boundary-based registration implemented in FSL FLIRT (6). The T1 images of each participant were registered to MNI152 standard space using 12-

parameter affine transformation and refined using non-linear registration with FSL FNIRT (10mm warp, 2mm resampling resolution; 3). Finally, we brought all participant-level R-fMRI images to 2mm MNI152 standard space by applying the R-fMRI to T1 and T1 to MNI152 transformations. All further analyses were conducted in MNI152 standard space.

2. Statistical power

We calculated the statistical sensitivity for the categorical analysis (ASD versus TD) and express this in terms of required effect size (quantified as critical Cohen's *d*) as well as for the continuous SRS-2 analysis where we express the required effect in terms of the critical minimal correlation. We determined (using G*Power; 7) that with our large sample we have –given an alpha of 0.05– 80% power to detect effects sizes as small as 0.295 in the categorical ASD (N=265) versus control group (N=138) analyses (two-tailed), and correlations as small as r=0.148 in the continuous SRS-2 analyses (N=358). These effects are of small to medium magnitude (8).

3. The autism trait-related associations investigated in the ASD and TD group separately

To ensure that the correlations with the Social Responsiveness Scale Second Edition (SRS-2; 9) observed across all participants in our continuous within- and between-network analyses did not simply represent artificial correlations induced by a general difference in the mean of the autism spectrum disorder (ASD) and typically developing (TD) control group, we investigated these associations in each group separately. To this end, we first extracted for every participant the mean Z-value of significant clusters identified in the within-network analysis or selected the connectivity value (i.e., Z-transformed Pearson correlation) for significant edges identified in the between-network analysis. We then computed correlations between these connectivity metrics and the SRS-2 scores in each group separately while correcting for potential confounding effects of scan site, age and sex. Table S1 and Figure S1 show that the observed correlations with the SRS-2 in our within-network analyses are also significant in the ASD group, indicating that our within-network findings are not induced by a general group difference. The correlations are not significant in the TD group, thus individuals with ASD are driving these effects. The absence of significant correlations in the TD group is not surprising given that this group only spans the lower end of the SRS-2 scale. It should however be noted that for our between-network analysis, the correlation of the SRS-2 with cerebellum–somatosensory network connectivity did not reach significance in the ASD group. This indicates that this finding might represent a more general difference in connectivity between both groups, which was indeed demonstrated in our case-control analysis. This association should therefore be interpreted with caution.

Networks	Correlation with SRS Whole sample *N=358*	Correlation with SRS TD only *N=117*	Correlation with SRS ASD only *N=241*
Within-network			
Salience anterior	**0.295*****	0.028	**0.229*****
Motor medial	**0.319*****	0.004	**0.295*****
OFC	**0.201*****	0.106	**0.223*****
Between-network			
OFC-motor lateral	**-0.229*****	-0.040	**-0.190****
OFC-DMN posterior	**-0.169*****	-0.097	**-0.201****
Cerebellum-somatosens.	**0.174*****	-0.044	0.082
Cerebellum-motor medial	**0.186*****	-0.062	**0.172****

Table S1. Correlations of the Social Responsiveness Scale Second edition (SRS-2) with within-network and between-network connectivity in the TD and ASD group separately. All correlations are corrected for site, sex and age. ***p*<0.01, ****p*<0.001.

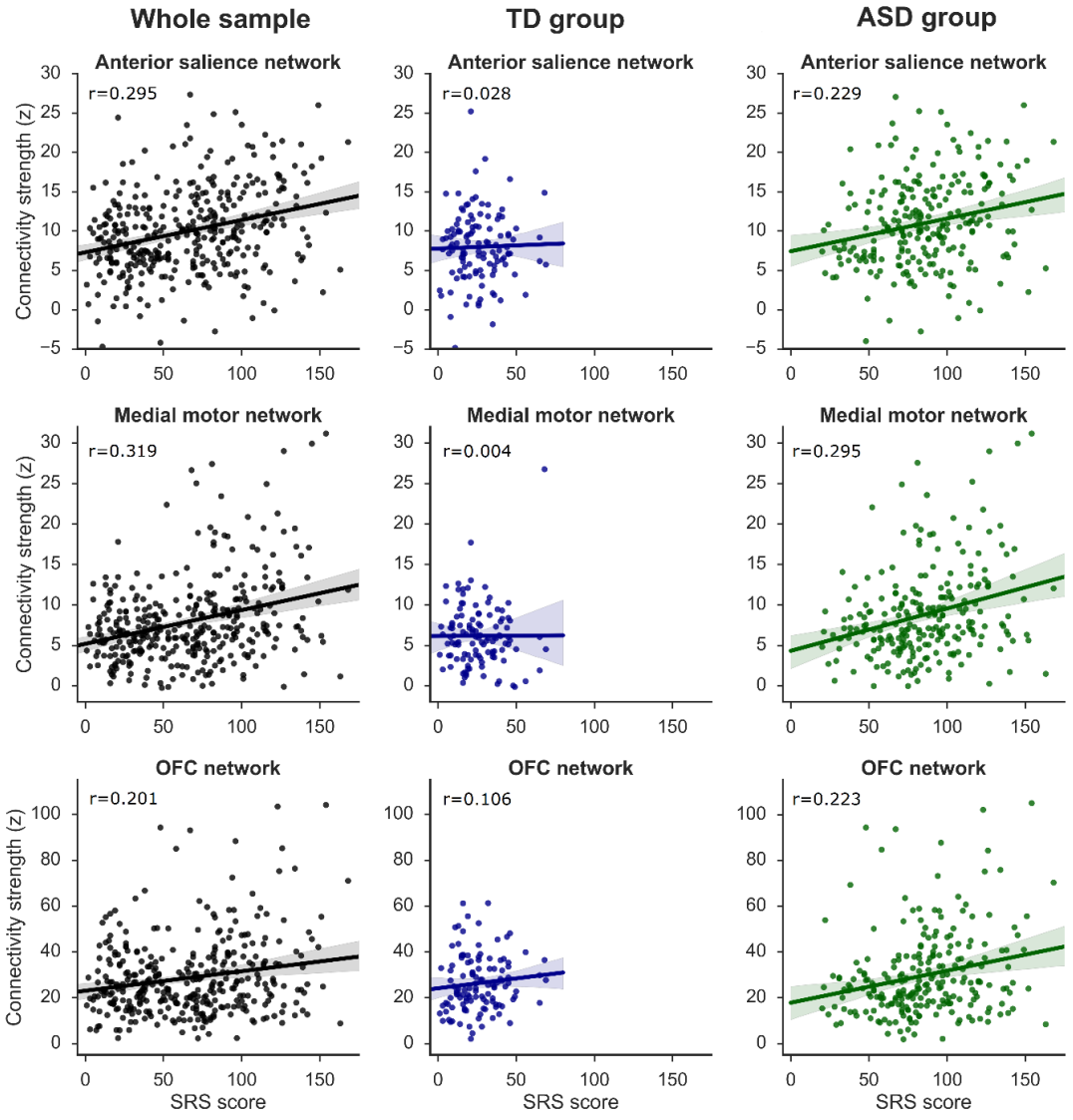


Figure S1. Correlations of the SRS-2 with within-network connectivity across the whole sample and in the TD and ASD group separately. OFC=orbitofrontal cortex.

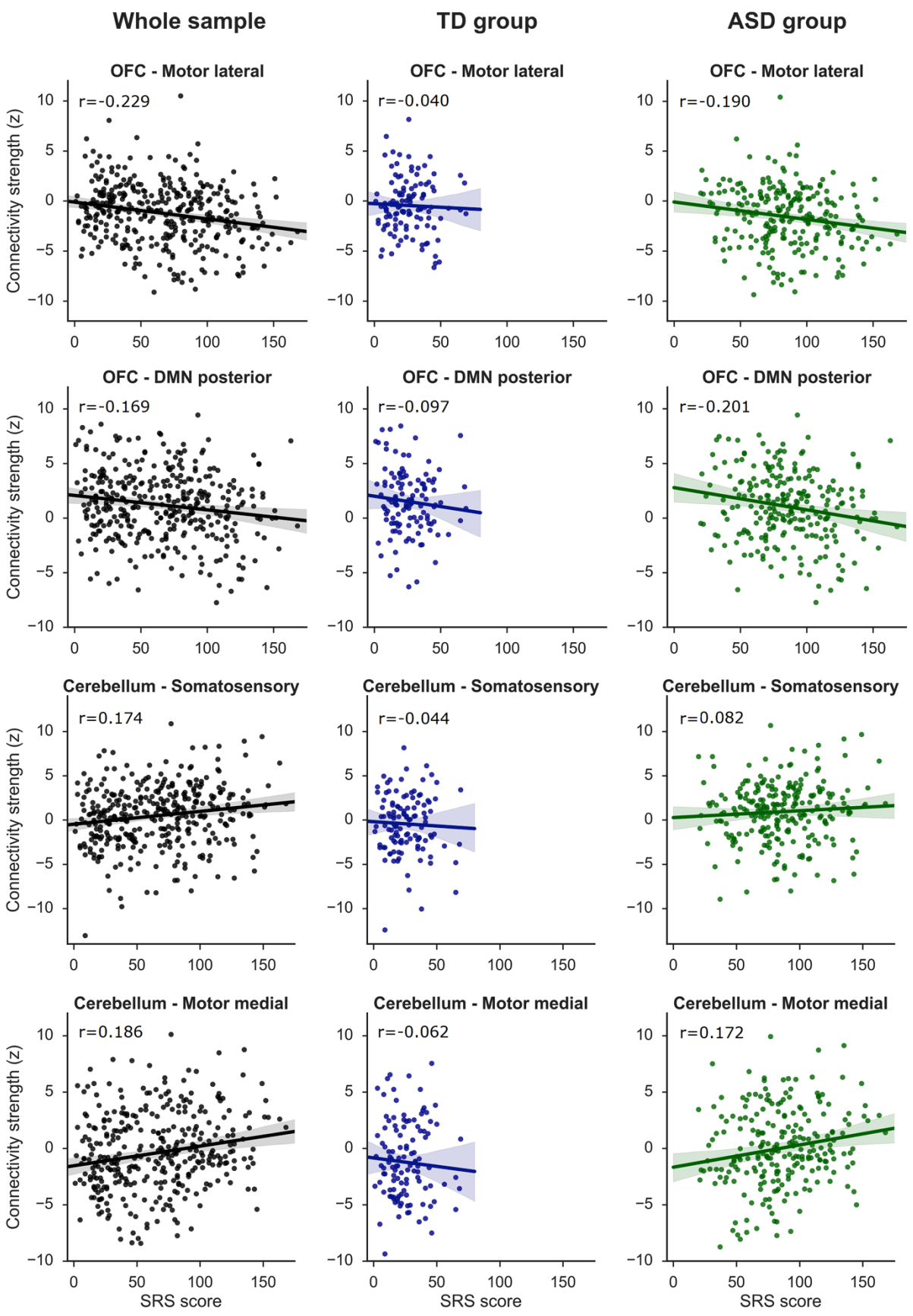


Figure S2. Correlations of the SRS-2 with between-network connectivity across the whole sample and in the TD and ASD group separately. OFC=orbitofrontal cortex, DMN=default mode network.

4. Boxplots of the significant case-control differences in between-network connectivity

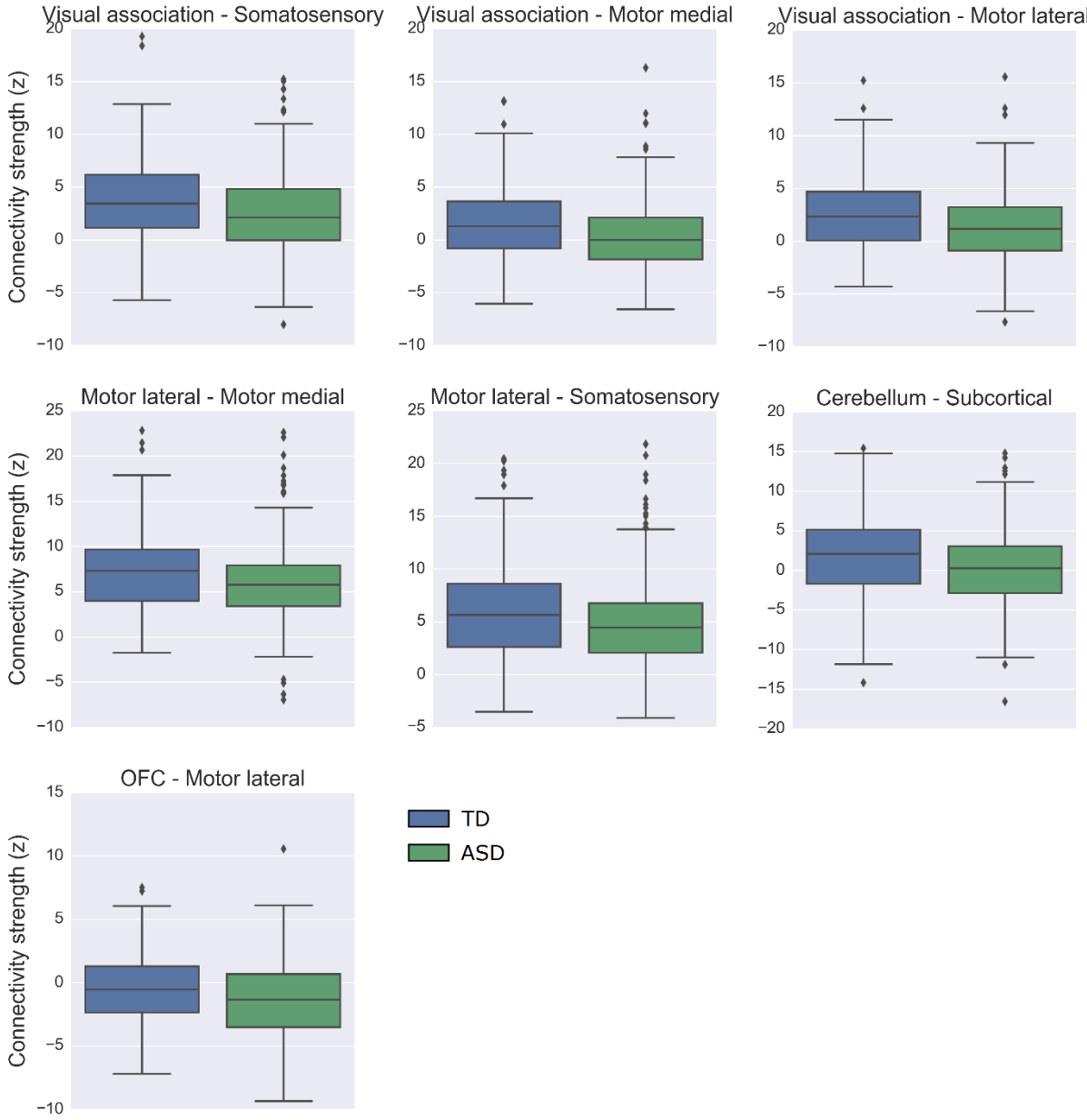


Figure S3a. Boxplots of edges for which connectivity was decreased in the ASD compared to TD group. OFC=orbitofrontal cortex.

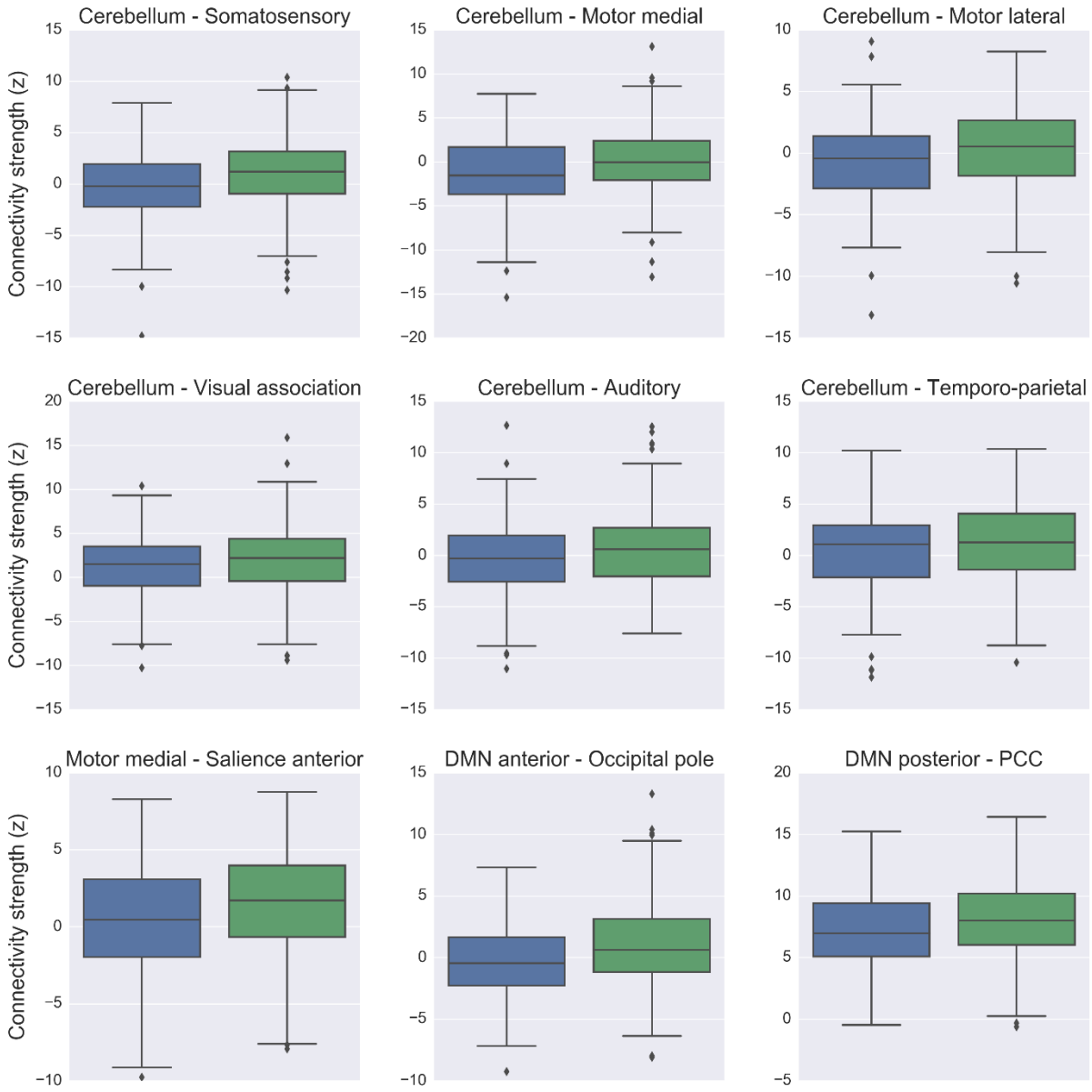


Figure S3b. Boxplots of edges for which connectivity was increased in the ASD compared to TD group. DMN=default mode network, PCC=posterior cingulate cortex.

5. Associations with ASD symptom domains

For all the significant ASD-related alterations in within and between-network connectivity identified in our analyses, we post-hoc examined associations with the different ASD symptom domains. To this end, we first extracted for every participant the mean Z-value of significant clusters identified in the within-network analysis or selected the connectivity value (i.e., Z-transformed Pearson correlation) for significant edges identified in the between-network analysis. We then computed correlations between these connectivity metrics and scores on the Social Communication and Interaction (SCI) and the Restrictive interests and Repetitive Behavior (RRB) subscales of the SRS-2, and scores on the Short Sensory Profile (SSP; 10), while correcting for potential confounding effects of scan site, age and sex. Higher SSP scores indicate less impairment; higher scores on the other scales indicate more impairment. These analyses revealed that functional connectivity within the three networks and functional connectivity for the four between-network edges significantly correlated with nearly all the investigated symptom measures (Table S2). For edges displaying case-control differences in between-network connectivity, correlations with the different ASD symptoms were lower than the correlations observed for connectivity alterations identified in the continuous SRS-2 analysis, yet significant associations were present for multiple edges (Table S3). Please note that the lower correlations with symptom scores for edges identified in the case-control between-network analysis is not surprising given that case-control differences do not necessarily depend on the within-group variances.

Networks	**SRS** *N=358*	**SRS SCI** *N=358*	**SRS RRB** *N=358*	**SSP** *N=207*
Within-network				
Salience anterior	**0.295*****	**0.297*****	**0.280*****	**-0.222****
Motor medial	**0.319*****	**0.310*****	**0.307*****	**-0.246*****
OFC	**0.201*****	**0.178*****	**0.170*****	**-0.165***
Between-network				
OFC-motor lateral	**-0.229*****	**-0.230****	**-0.202*****	0.095
OFC-DMN posterior	**-0.169*****	**-0.156****	**-0.131***	**0.169***
Cerebellum-somatosens.	**0.174*****	**0.168****	**0.212*****	**-0.170***
Cerebellum-motor medial	**0.186*****	**0.171****	**0.210*****	**-0.163***

Table S2. Correlations of continuous connectivity alterations with ASD symptoms. The correlation with the SRS represents the original correlation with the SRS-2 as identified in the main analysis. All correlations are corrected for site, sex and age. **p*<0.05, ***p*<0.01, ****p*<0.001. SRS=Social Responsiveness Scale Second Edition (SRS-2; 10), SRS SCI=Social Communication and Interaction subscale of the SRS-2, SRS RRB=Restricted interests and Repetitive Behavior subscale of the SRS-2. SSP=Short Sensory Profile (11). The relatively low number of participants with SSP total scores available is explained by the fact that the SSP questionnaire allowed parents to answer with "not applicable" in cases where they were not able to observe a particular behavior or this type of behavior was not applicable. If an item was responded to in such a way, the response to this item needed to be dismissed and total scores for these participants were not included in this analysis.

Network 1	Network 2	SRS *N=358*	SRS SCI *N=358*	SRS RRB *N=358*	SSP *N=213*
Edges of decreased connectivity in the ASD group					
Visual association	Somatosensory	**-0.126***	**-0.127***	-0.075	0.102
Visual association	Motor medial	**-0.134***	**-0.131***	**-0.119***	**0.162***
Visual association	Motor lateral	**-0.145****	**-0.136***	**-0.135***	0.128
Motor medial	Motor lateral	**-0.105***	-0.105	-0.101	0.1325
Motor lateral	Somatosensory	-0.024	-0.022	-0.028	0.105
Cerebellum	Subcortical	-0.062	-0.076	-0.013	-0.048
OFC	Motor lateral	**-0.229*****	**-0.227*****	**-0.199*****	0.097
Edges of increased connectivity in the ASD group					
Cerebellum	Somatosensory	**0.174*****	**0.172*****	**0.216*****	**-0.162***
Cerebellum	Motor medial	**0.186*****	**0.170*****	**0.209*****	**-0.155***
Cerebellum	Motor lateral	0.098	**0.113***	0.083	-0.093
Cerebellum	Visual association	0.083	0.070	**0.112***	-0.098
Cerebellum	Auditory	0.079	0.094	0.055	-0.012
Cerebellum	Temporo-parietal	0.035	0.035	0.019	-0.011
Motor medial	Salience anterior	**0.143****	**0.149****	**0.131***	**-0.150***
DMN anterior	Occipital pole	**0.136***	**0.121***	**0.119***	-0.123
DMN posterior	PCC	0.030	0.027	0.023	0.019

Table S3. Correlations of between-network connectivity with ASD symptoms. All correlations are corrected for site, sex and age. *p<0.05, **p<0.01, ***p<0.001. SRS=Social Responsiveness Scale Second Edition (SRS-2; 10), SRS SCI=Social Communication and Interaction subscale of the SRS-2, SRS RRB=Restricted interests and Repetitive Behavior subscale of the SRS-2. SSP=Short Sensory Profile (11). The relatively low number of participants with SSP total scores available is explained by the fact that the SSP questionnaire allowed parents to answer with "not applicable" in cases where they were not able to observe a particular behavior or this type of behavior was not applicable. If an item was responded to in such a way, the response to this item needed to be dismissed and total scores for these participants were not included in this analysis.

6. Developmental effects

In light of potential development effects, we performed two types of analyses. First, we investigated post-hoc whether the ASD-related alterations in within- or between-network connectivity as revealed by our analyses were present in each of the following age groups: children (6.9-11 years), adolescents (12-17 years) and adults (18-30). To this end, we computed –separately for each age group– correlations between SRS-2 scores and functional connectivity (i.e., the mean Z-value of significant clusters for within-network connectivity or Z-transformed Pearson correlations for between-network connectivity) for continuous ASD-related alterations and Cohen's D effect sizes for case-control differences in between-network connectivity. These analyses aimed to qualitatively confirm that effects within each investigated age-group adhered to the same direction as our main findings, rather than demonstrating that effects within subgroups remained significant, as splitting into smaller groups will affect statistical power. This analysis revealed that the continuous ASD-related alterations in functional connectivity were present across all the investigated age-groups (Table S4). Case-control differences in between-network connectivity were clearly present in adults and adolescents, however in children effect sizes for a few edges were very small and/or were in the opposite direction as the main effect (Table S5), suggesting that connectivity abnormalities for these between-network connections are not yet present in childhood but might develop during adolescence in ASD. This warrants further investigation into the development of these between-network connections in ASD.

Networks	**SRS corr. Whole sample** *N=358*	**SRS corr. Adults** *N=143*	**SRS corr. Adolescents** *N=151*	**SRS corr. Children** *N=64*
Within-network				
Salience anterior	**0.295*****	**0.290*****	**0.339*****	0.189
Motor medial	**0.319*****	**0.374*****	**0.300*****	**0.250***
OFC	**0.201*****	**0.243****	**0.172***	0.175
Between-network				
OFC-motor lateral	**-0.229*****	**-0.266****	**-0.188***	-0.244
OFC-DMN posterior	**-0.169*****	-0.121	**-0.197***	-0.195
Cerebellum-somatosens.	**0.174*****	0.154	0.140	**0.304***
Cerebellum-motor medial	**0.186*****	**0.193***	0.135	**0.257***

Table S4. Correlations with the SRS-2 across different age groups. Age ranges: children: 6.9-11 years, adolescents: 12-17 years, adults: 18-30 years. SRS corr=correlation with the SRS-2. **p*<0.05, ***p*<0.01, ****p*<0.001.

Network 1	Network 2	Cohen's D Whole sample N_{TD}=138 N_{ASD}=265	Cohen's D Adults N_{TD}=57 N_{ASD}=103	Cohen's D Adolescents N_{TD}=59 N_{ASD}=109	Cohen's D Children N_{TD}=22 N_{ASD}=53
Edges of decreased connectivity in the ASD group					
Visual association	Somatosensory	**-0.3116*** **	-0.3283	**-0.3896***	-0.2259
Visual association	Motor medial	**-0.3819*** **	**-0.4442****	**-0.3612***	-0.1994
Visual association	Motor lateral	**-0.3801*** **	**-0.3796***	**-0.5489*** **	-0.2086
Motor medial	Motor lateral	**-0.3215*** **	-0.2976	**-0.4562****	0.1397
Motor lateral	Somatosensory	**-0.3022*** **	-0.2733	-0.3306	-0.0813
Cerebellum	Subcortical	**-0.3520*** **	-0.3067	-0.2396	-0.4518
OFC	Motor lateral	**-0.3470*** **	**-0.4068***	-0.2389	**-0.5424***
Edges of increased connectivity in the ASD group					
Cerebellum	Somatosensory	**0.3526*** **	**0.4017***	0.2141	0.4298
Cerebellum	Motor medial	**0.3417*** **	**0.3844***	0.2032	0.4431
Cerebellum	Motor lateral	**0.3480*** **	**0.4313****	0.1253	0.2210
Cerebellum	Visual association	**0.3127*** **	**0.4151***	0.1181	-0.0187
Cerebellum	Auditory	**0.3005*** **	0.2573	0.0533	**0.5479****
Cerebellum	Temporo-parietal	**0.2943*** **	**0.4076***	-0.0243	0.2464
Motor medial	Salience	**0.3256*** **	0.2214	**0.3914***	0.4782
DMN anterior	Occipital pole	**0.3659*** **	0.3148	**0.5654*** **	-0.1353
DMN posterior	PCC	**0.3313*** **	**0.4464****	0.2894	0.0876

Table S5. Effect sizes of case-control differences in between-network connectivity across different age groups. Age ranges: children: 6.9-11 years, adolescents: 12-17 years, adults: 18-30 years. *p<0.05, **p<0.01, ***p<0.001.

In addition, we repeated our main analyses in children, adolescents and adults separately to investigate whether additional ASD-related decreases or increases in within or between-network connectivity were revealed by investigating each age-group independently. This analysis did not reveal any other significant (p<0.0025) ASD-related changes in within- or between-network connectivity in addition to the connectivity alterations that were already observed in our main analyses.

7. Sensitivity analyses

For all the significant ASD-related alterations in within and between-network connectivity identified in our analyses, we also conducted post-hoc sensitivity analyses to rule out that significant connectivity alterations were driven by head motion, informant (parent or self-report SRS score for the continuous analyses), sex, scan site, IQ, medication use, or comorbidity with ADHD. All these analyses aimed to qualitatively confirm that effects within each investigated subgroup adhered to the same direction as our main findings (e.g., SRS-related increases in within-network connectivity or increased/decreased between-network connectivity in the ASD compared to TD group), rather than demonstrating that effects within subgroups remained significant, as splitting into smaller groups will affect statistical power. We provide correlations between SRS-2 scores and functional connectivity (i.e., the mean Z-value of significant clusters for within-network connectivity or Z-transformed Pearson correlations for between-network connectivity) for continuous ASD-related alterations and Cohen's D effect sizes for case-control differences in between-network connectivity, to give a concise overview of the results for the many post-hoc tests that were conducted.

7.1 Head motion

We thoroughly corrected for secondary head-motion related artifacts, by applying ICA-AROMA (4) a novel ICA-based method demonstrated to remove head motion-related artifacts with high accuracy while preserving signal of interest (4, 5). In addition, we also excluded participants with high head motion (root mean squared of the mean frame wise displacement (meanFD)>0.5) from our analyses. Applying ICA-AROMA mitigates the need to additionally correct for head motion by for example including motion-related variables in the statistical model. Nevertheless, to rule out that the ASD-related connectivity alterations in our analyses were induced by potential residual effects of head motion, we repeated our main analyses and included a covariate for head motion (meanFD) in our statistical models. Second, we investigated ASD-related connectivity alterations in subgroups of participants displaying minimal head motion (meanFD<0.2) and very minimal head motion (meanFD<0.1). Correlations with the SRS-2 and effects sizes in the minimal head motion groups and in the analysis correcting for meanFD were comparable with the effects observed in our main analyses, indicating that our findings were not induced by head motion.

Networks	SRS corr. Whole sample *N=358*	SRS corr. Whole sample +meanFD corrected *N=358*	SRS corr. meanFD<0.2 *N=324*	SRS corr. meanFD<0.1 *N=255*
Within-network				
Salience anterior	0.295	0.256	0.320	0.275
Motor medial	0.319	0.264	0.239	0.170
OFC	0.201	0.137	0.171	0.141
Between-network				
OFC-motor lateral	-0.229	-0.212	-0.222	-0.267
OFC-DMN posterior	-0.169	-0.144	-0.161	-0.197
Cerebellum-somatosens.	0.174	0.172	0.171	0.180
Cerebellum-motor medial	0.186	0.203	0.177	0.185

Table S6. Correlations with the SRS-2 when correcting for meanFD, and under different motion-thresholds. Corrected for scan site, sex and age. MeanFD=mean frame wise displacement.

Network 1	Network 2	Cohen's D Whole sample N_{TD}=138 N_{ASD}=265	Cohen's D Whole sample +meanFD corrected N_{TD}=138 N_{ASD}=265	Cohen's D meanFD <0.2 N_{TD}=132 N_{ASD}=231	Cohen's D meanFD <0.1 N_{TD}=110 N_{ASD}=170
Edges of decreased connectivity in the ASD group					
Visual association	Somatosensory	-0.3116	-0.3148	-0.3080	-0.4121
Visual association	Motor medial	-0.3819	-0.3509	-0.3704	-0.5390
Visual association	Motor lateral	-0.3801	-0.3573	-0.3778	-0.4738
Motor medial	Motor lateral	-0.3215	-0.2705	-0.3167	-0.3605
Motor lateral	Somatosensory	-0.3022	-0.2709	-0.3073	-0.4050
Cerebellum	Subcortical	-0.3520	-0.3496	-0.3618	-0.5244
OFC	Motor lateral	-0.3470	-0.3213	-0.3928	-0.4449
Edges of increased connectivity in the ASD group					
Cerebellum	Somatosensory	0.3526	0.3561	0.3626	0.3937
Cerebellum	Motor medial	0.3417	0.3684	0.3520	0.3607
Cerebellum	Motor lateral	0.3480	0.3488	0.3585	0.4088
Cerebellum	Visual association	0.3127	0.2650	0.3149	0.3551
Cerebellum	Auditory	0.3005	0.3388	0.3264	0.3281
Cerebellum	Temporo-parietal	0.2943	0.3442	0.3253	0.4025
Motor medial	Salience	0.3256	0.3180	0.3719	0.3800
DMN anterior	Occipital pole	0.3659	0.3238	0.3581	0.3412
DMN posterior	PCC	0.3313	0.3236	0.3317	0.3674

Table S7. Effect sizes of case-control differences in between-network connectivity when correcting for meanFD, and under different motion-thresholds. Corrected for site, sex and age. MeanFD=mean frame wise displacement.

7.2 SRS informant

In our continuous analyses, we used the parent-report SRS-2 score when available, for the remaining participants we used the self-report SRS-2 score. The parent-report SRS-2 was administered to the parents of all participants, except for adult TD participants. The self-report SRS-2 was completed by adult and adolescent TD participants and adult and adolescent ASD patients. To investigate the influence of SRS informant (parent or self), we calculated the correlation of the SRS-2 score with within- and between-network connectivity separately for the parent-report and self-report. This analysis demonstrates that the SRS-related increases in within-network connectivity and the SRS-related alterations in between-network connectivity are present for both the parent-report and self-report.

Networks	**SRS corr. Whole sample** *N=358*	**SRS corr. Parent-report** *N=282*	**SRS corr. Self-report** *N=228*
Within-network			
Salience anterior	0.295	0.297	0.323
Motor medial	0.319	0.321	0.257
OFC	0.201	0.234	0.190
Between-network			
OFC-motor lateral	-0.229	-0.212	-0.161
OFC-DMN posterior	-0.169	-0.215	-0.042
Cerebellum-somatosens.	0.174	0.136	0.221
Cerebellum-motor medial	0.186	0.160	0.206

Table S8. Correlations with the SRS-2 based on parent-report and self-report. Corrected for scan site, sex and age.

7.3 Sex

We subdivided our sample in male and female participants to investigate the influence of biological sex on our findings. Similar correlations with the SRS-2 and effects sizes were present in males and females, indicating that biological sex did not influence our findings.

Networks	**SRS corr. Whole sample** *N=358*	**SRS corr. Males** *N=248*	**SRS corr. Females** *N=110*
Within-network			
Salience anterior	0.295	0.294	0.298
Motor medial	0.319	0.323	0.317
OFC	0.201	0.237	0.105
Between-network			
OFC-motor lateral	-0.229	-0.207	-0.276
OFC-DMN posterior	-0.169	-0.172	-0.160
Cerebellum-somatosens.	0.174	0.176	0.173
Cerebellum-motor medial	0.186	0.208	0.131

Table S9. Correlations with the SRS-2 in males and females. Corrected for scan site and age.

Network 1	**Network 2**	**Cohen's D Whole sample** N_{TD}=138 N_{ASD}=265	**Cohen's D Males** N_{TD}=90 N_{ASD}=194	**Cohen's D Females** N_{TD}=48 N_{ASD}=71
Edges of decreased connectivity in the ASD group				
Visual association	Somatosensory	-0.3116	-0.3324	-0.2620
Visual association	Motor medial	-0.3819	-0.4853	-0.2528
Visual association	Motor lateral	-0.3801	-0.3665	-0.4120
Motor medial	Motor lateral	-0.3215	-0.4009	-0.1992
Motor lateral	Somatosensory	-0.3022	-0.2926	-0.3653
Cerebellum	Subcortical	-0.3520	-0.2752	-0.5041
OFC	Motor lateral	-0.3470	-0.3352	-0.3164
Edges of increased connectivity in the ASD group				
Cerebellum	Somatosensory	0.3526	0.3183	0.5316
Cerebellum	Motor medial	0.3417	0.3537	0.3389
Cerebellum	Motor lateral	0.3480	0.3615	0.3099
Cerebellum	Visual association	0.3127	0.3018	0.3710
Cerebellum	Auditory	0.3005	0.3369	0.3130
Cerebellum	Temporo-parietal	0.2943	0.2055	0.5480
Motor medial	Salience	0.3256	0.3087	0.3904
DMN anterior	Occipital pole	0.3659	0.3831	0.3686
DMN posterior	PCC	0.3313	0.3556	0.3580

Table S10. Effect sizes of case-control differences in between-network connectivity in males and females. Corrected for scan site and age.

7.4 Scan site

To investigate the influence of scan site our findings, we computed correlations with the SRS-2 and effect sizes for case-control differences in between-network connectivity for each of the five scan sites separately. There is variability across scan sites, but the direction of correlations with the SRS-2 and effects sizes is the same as observed in the main analysis, apart from a few exceptions. However, given that there are no systematic, scan site-dependent alterations in connectivity, we can conclude that our findings are not dependent on scan site.

Networks	**SRS corr. Whole sample** *N=358*	**SRS corr. KCL** *N=113*	**SRS corr. RUNMC** *N=109*	**SRS corr. UCAM** *N=50*	**SRS corr. UMCU** *N=44*	**SRS corr. CIMH** *N=42*
Within-network						
Salience anterior	0.295	0.359	0.205	0.376	0.082	0.209
Motor medial	0.319	0.258	0.189	0.518	0.146	0.215
OFC	0.201	0.247	0.116	0.493	0.078	-0.010
Between-network						
OFC-motor lateral	-0.229	-0.221	-0.091	-0.368	-0.206	-0.333
OFC-DMN posterior	-0.169	-0.241	-0.080	-0.358	-0.169	-0.130
Cerebellum-somatosens.	0.174	0.248	0.142	0.126	0.011	0.160
Cerebellum-motor medial	0.186	0.267	0.176	0.072	0.098	0.225

Table S11. Correlations with the SRS-2 across the different scan sites. Corrected for sex and age. KCL=King's College London, RUNMC=Radboud University Nijmegen Medical Center, UCAM=University of Cambridge, UMCU=University Medical Center Utrecht, CIMH=Central Institute of Mental Health Mannheim.

Network 1	Network 2	Cohen's D KCL N_{TD}=38 N_{ASD}=83	Cohen's D RUNMC N_{TD}=35 N_{ASD}=81	Cohen's D UCAM N_{TD}=16 N_{ASD}=44	Cohen's D UMCU N_{TD}=25 N_{ASD}=33	Cohen's D CIMH N_{TD}=24 N_{ASD}=24
Edges of decreased connectivity in the ASD group						
Visual association	Somatosensory	-0.0249	-0.3801	0.2260	-0.6380	-1.3052
Visual association	Motor medial	-0.3256	-0.2294	-0.0928	-0.6067	-1.2632
Visual association	Motor lateral	-0.3400	-0.4228	-0.1084	0.0797	-1.1602
Motor medial	Motor lateral	-0.0067	-0.5747	-0.3314	-0.0516	-1.0243
Motor lateral	Somatosensory	-0.0110	-0.5603	-0.2022	0.0580	-1.3892
Cerebellum	Subcortical	-0.2233	-0.2083	-0.2460	-0.5196	-0.7277
OFC	Motor lateral	-0.4590	-0.0479	-0.3431	-0.4178	-0.7379
Edges of increased connectivity in the ASD group						
Cerebellum	Somatosensory	0.4731	0.1113	0.1017	0.3820	0.7801
Cerebellum	Motor medial	0.4694	0.1604	0.0599	0.3351	0.6235
Cerebellum	Motor lateral	0.2284	0.1733	0.5358	0.6026	0.5435
Cerebellum	Visual association	0.4636	0.1526	0.6074	0.0669	0.3421
Cerebellum	Auditory	0.2581	0.0880	-0.0438	0.9960	0.9606
Cerebellum	Temporo-parietal	0.3341	0.0379	-0.0636	0.8023	0.5009
Motor medial	Salience	-0.4784	0.5457	1.0686	0.7118	0.6350
DMN anterior	Occipital pole	0.3264	0.3761	0.7547	0.4899	0.1281
DMN posterior	PCC	0.4620	0.1706	0.7155	0.6916	0.0331

Table S12. Effect sizes of case-control differences in between-network connectivity across the different scan sites. Corrected for sex and age. KCL=King's College London, RUNMC=Radboud University Nijmegen Medical Center, UCAM=University of Cambridge, UMCU=University Medical Center Utrecht, CIMH=Central Institute of Mental Health Mannheim.

7.5 IQ

To investigate the influence of IQ on our findings, we first repeated our main analyses and included a covariate for IQ. This analysis resulted in similar correlations with the SRS-2 and effect sizes as observed in our main analysis. In addition, we divided our sample in subjects with an IQ below and above 100. Correlations with the SRS-2 and effects sizes were present in both subgroups, however most effects were more pronounced in the group with a lower IQ. This might be related to the on average higher symptom severity in ASD individuals with a lower IQ.

Networks	**SRS corr. Whole sample** *N=358*	**SRS corr. Whole sample +corrected for IQ** *N=358*	**SRS corr. IQ<100** *N=117*	**SRS corr. IQ>100** *N=229*
Within-network				
Salience anterior	0.295	0.277	0.291	0.278
Motor medial	0.319	0.297	0.487	0.185
OFC	0.201	0.184	0.262	0.135
Between-network				
OFC-motor lateral	-0.229	-0.216	-0.219	-0.219
OFC-DMN posterior	-0.169	-0.159	-0.244	-0.122
Cerebellum-somatosens.	0.174	0.172	0.162	0.168
Cerebellum-motor medial	0.186	0.198	0.180	0.193

Table S13. Correlations with the SRS-2 when correcting for IQ, and in participants with an IQ below and above 100. Corrected for scan site, sex and age.

Network 1	Network 2	Cohen's D Whole sample N_{TD}=138 N_{ASD}=265	Cohen's D Whole sample +corrected for IQ N_{TD}=138 N_{ASD}=265	Cohen's D IQ<100 N_{TD}=35 N_{ASD}=99	Cohen's D IQ>100 N_{TD}=103 N_{ASD}=166
Edges of decreased connectivity in the ASD group					
Visual association	Somatosensory	-0.3116	-0.3234	-0.6079	-0.2148
Visual association	Motor medial	-0.3819	-0.4063	-0.7165	-0.2867
Visual association	Motor lateral	-0.3801	-0.4065	-0.7474	-0.2846
Motor medial	Motor lateral	-0.3215	-0.3198	-0.6453	-0.2097
Motor lateral	somatosensory	-0.3022	-0.3108	-0.7912	-0.1521
Cerebellum	Subcortical	-0.3520	-0.3752	-0.4102	-0.3963
OFC	Motor lateral	-0.3470	-0.3153	-0.5097	-0.2576
Edges of increased connectivity in the ASD group					
Cerebellum	Somatosensory	0.3526	0.3386	0.3316	0.3356
Cerebellum	Motor medial	0.3417	0.3506	0.3651	0.3400
Cerebellum	Motor lateral	0.3480	0.3519	0.6006	0.2515
Cerebellum	Visual association	0.3127	0.2905	0.6833	0.1511
Cerebellum	Auditory	0.3005	0.3270	0.3252	0.3252
Cerebellum	Temporo-parietal	0.2943	0.3142	0.6530	0.2014
Motor medial	Salience	0.3256	0.3255	0.5045	0.2920
DMN anterior	Occipital pole	0.3659	0.3589	0.1670	0.3819
DMN posterior	PCC	0.3313	0.3289	0.2505	0.3581

Table S14. Effects sizes of case-control differences in between-network connectivity when correcting for IQ, and in participants with an IQ below and above 100. Corrected for scan site, sex and age.

7.6 Medication use

Participants with ASD were included independent of medication use. To test for the influence of medication on our findings we divided our sample in participants that did not use medication and participants that used medication prescribed for behavioral or neurological problems, and computed the correlations with the SRS-2 and effect sizes for case-control differences for both groups separately. This analysis revealed correlations with the SRS-2 and effect sizes in both groups that are overall comparable to those observed in the main analyses, indicating that our findings were not induced by medication use.

Networks	**SRS corr. Whole sample** *N=358*	**SRS corr. No medication** *N=262*	**SRS corr. Medication** *N=68*
Within-network			
Salience anterior	0.295	0.297	0.229
Motor medial	0.319	0.358	0.217
OFC	0.201	0.241	0.183
Between-network			
OFC-motor lateral	-0.229	-0.202	-0.265
OFC-DMN posterior	-0.169	-0.184	-0.340
Cerebellum-somatosens.	0.174	0.190	0.147
Cerebellum-motor medial	0.186	0.190	0.239

Table S15. Correlations with the SRS in participants without and with medication. Corrected for scan site, sex and age.

Network 1	Network 2	Cohen's D Whole sample N_{TD}=138 N_{ASD}=265	Cohen's D no medication N_{TD}=138 N_{ASD}=170	Cohen's D on medication N_{TD}=138 N_{ASD}=68
Edges of decreased connectivity in the ASD group				
Visual association	Somatosensory	-0.3116	-0.2873	-0.3053
Visual association	Motor medial	-0.3819	-0.3173	-0.5398
Visual association	Motor lateral	-0.3801	-0.2694	-0.6293
Motor medial	Motor lateral	-0.3215	-0.3552	-0.2797
Motor lateral	Somatosensory	-0.3022	-0.2778	-0.3179
Cerebellum	Subcortical	-0.3520	-0.3029	-0.3843
OFC	Motor lateral	-0.3470	-0.2665	-0.4423
Edges of increased connectivity in the ASD group				
Cerebellum	Somatosensory	0.3526	0.3799	0.2449
Cerebellum	Motor medial	0.3417	0.3594	0.3055
Cerebellum	Motor lateral	0.3480	0.4034	0.2620
Cerebellum	Visual association	0.3127	0.2468	0.3904
Cerebellum	Auditory	0.3005	0.3618	0.1077
Cerebellum	Temporo-parietal	0.2943	0.3434	0.1326
Motor medial	Salience	0.3256	0.2364	0.4543
DMN anterior	Occipital pole	0.3659	0.2918	0.5282
DMN posterior	PCC	0.3313	0.3420	0.2754

Table S16. Effect sizes of case-control differences in between-network connectivity comparing the control group with an ASD group without and with medication. Corrected for scan site, sex and age.

7.7 ADHD comorbidity

We did not exclude ASD participants with an ADHD comorbidity given that ADHD prevalently co-occurs with ASD (11). To investigate the influence of ADHD symptoms on our findings, we first repeated our main analyses and included a covariate for the total number of ADHD symptoms derived from the DSM-5 ADHD rating scale (12). Scores from the ADHD rating scale were available for 352 of the 403 participants in the categorical analysis and for 347 of the 358 participants in the continuous analysis. This analysis resulted in overall similar correlations with the SRS-2 and effect sizes as observed in our main analysis, although the drop in the correlation of SRS scores with the two cerebellar edges is evident, potentially indicating that connectivity of these two edges might also be related to ADHD symptoms. In addition, we defined two groups of participants, one group without ADHD comorbidity having ≤4 inattentive and ≤4 hyperactive/impulsive symptoms and one group which likely has ADHD comorbidity, consisting of participants displaying ≥6 symptoms in the inattention and/or hyperactivity/impulsivity domain. We computed the correlations with the SRS-2 and effect sizes for case-control differences in between-network connectivity for both groups separately. This analysis revealed correlations with the SRS-2 and effect sizes in both groups that were comparable to those observed in the main analyses.

Networks	**SRS corr. Whole sample** *N=358*	**SRS corr. Whole sample +corrected for total ADHD symptoms** *N=347*	**SRS corr. No ADHD comorbidity** *N=219*	**SRS corr. Potential ADHD comorbidity** *N=96*
Within-network				
Salience anterior	0.295	0.222	0.328	0.161
Motor medial	0.319	0.239	0.215	0.247
OFC	0.201	0.196	0.138	0.230
Between-network				
OFC-motor lateral	-0.229	-0.208	-0.129	-0.233
OFC-DMN posterior	-0.169	-0.155	-0.169	-0.182
Cerebellum-somatosens.	0.174	0.065	0.093	0.142
Cerebellum-motor medial	0.186	0.087	0.193	0.203

Table S17. Correlations with the SRS when correcting for the number of ADHD symptoms and in participants without and with ADHD comorbidity. Corrected for scan site, sex and age.

Network 1	Network 2	Cohen's D Whole sample N_{TD}=138 N_{ASD}=265	Cohen's D Whole sample +corrected for ADHD symptoms N_{TD}=109 N_{ASD}=237	Cohen's D No ADHD comorbidity N_{TD}=109 N_{ASD}=127	Cohen's D ASD with potential ADHD comorbidity N_{TD}=109 N_{ASD}=90
Edges of decreased connectivity in the ASD group					
Visual association	Somatosensory	-0.3116	-0.3200	-0.2361	-0.4697
Visual association	Motor medial	-0.3819	-0.3678	-0.2518	-0.5092
Visual association	Motor lateral	-0.3801	-0.4316	-0.2890	-0.3378
Motor medial	Motor lateral	-0.3215	-0.2281	-0.2105	-0.1834
Motor lateral	Somatosensory	-0.3022	-0.2328	-0.1379	-0.2668
Cerebellum	Subcortical	-0.3520	-0.3703	-0.4319	-0.2796
OFC	Motor lateral	-0.3470	-0.2766	-0.3877	-0.4330
Edges of increased connectivity in the ASD group					
Cerebellum	Somatosensory	0.3526	0.3631	0.3294	0.6123
Cerebellum	Motor medial	0.3417	0.3307	0.2557	0.5555
Cerebellum	Motor lateral	0.3480	0.4165	0.3318	0.4980
Cerebellum	Visual association	0.3127	0.2578	0.2503	0.3525
Cerebellum	Auditory	0.3005	0.3276	0.3923	0.2445
Cerebellum	Temporo-parietal	0.2943	0.2258	0.3738	0.1465
Motor medial	Salience	0.3256	0.2943	0.2632	0.3686
DMN anterior	Occipital pole	0.3659	0.3490	0.3970	0.3472
DMN posterior	PCC	0.3313	0.0903	0.3624	0.1330

Table S18. Effect sizes of case-control differences in between-network connectivity when correcting for the number of ADHD symptoms and when comparing the control group with an ASD group without and with ADHD comorbidity. Corrected for scan site, sex and age. There were also six TD participants with a high number of ADHD symptoms, but these participants were excluded from this analysis.

7.8 Comorbidity with anxiety

We did not exclude ASD participants with an anxiety comorbidity given that anxiety prevalently co-occurs with ASD (11). To investigate the influence of anxiety symptoms on our findings, we repeated our main analyses and included a covariate for anxiety severity derived from the Beck Anxiety Inventory (BAI; 13), a questionnaire consisting of 21 items with scores ranging from 0 to 63. BAI total scores were available for 309 of the 403 participants in the categorical analysis and for 305 of the 308 participants in the continuous analysis. This analysis revealed correlations with the SRS-2 and effect sizes in both groups that were comparable to those observed in the main analyses, indicating that our findings were not induced by anxiety symptoms.

Networks	**SRS corr.** **Whole sample** *N=358*	**SRS corr.** **Whole sample** **+corrected for total anxiety scores** *N=305*
Within-network		
Salience anterior	0.295	0.280
Motor medial	0.319	0.284
OFC	0.201	0.164
Between-network		
OFC-motor lateral	-0.229	-0.184
OFC-DMN posterior	-0.169	-0.136
Cerebellum-somatosens.	0.174	0.159
Cerebellum-motor medial	0.186	0.178

Table S19. Correlations with the SRS when correcting for anxiety scores. Corrected for scan site, sex and age.

Network 1	Network 2	Cohen's D Whole sample N_{TD}=138 N_{ASD}=265	Cohen's D Whole sample +corrected for anxiety symptoms N_{TD}=105 N_{ASD}=204
Edges of decreased connectivity in the ASD group			
Visual association	Somatosensory	-0.3116	-0.3548
Visual association	Motor medial	-0.3819	-0.3236
Visual association	Motor lateral	-0.3801	-0.4222
Motor medial	Motor lateral	-0.3215	-0.2473
Motor lateral	Somatosensory	-0.3022	-0.2578
Cerebellum	Subcortical	-0.3520	-0.3292
OFC	Motor lateral	-0.3470	-0.2896
Edges of increased connectivity in the ASD group			
Cerebellum	Somatosensory	0.3526	0.4181
Cerebellum	Motor medial	0.3417	0.3746
Cerebellum	Motor lateral	0.3480	0.4335
Cerebellum	Visual association	0.3127	0.2794
Cerebellum	Auditory	0.3005	0.3083
Cerebellum	Temporo-parietal	0.2943	0.2171
Motor medial	Salience	0.3256	0.3172
DMN anterior	Occipital pole	0.3659	0.3437
DMN posterior	PCC	0.3313	0.0722

Table S20. Effect sizes of case-control differences in between-network connectivity when correcting for anxiety scores. Corrected for scan site, sex and age.

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